EFFECTS OF HEAD-UP TILT ON MEAN ARTERIAL PRESSURE, HEART RATE, AND REGIONAL CARDIAC OUTPUT DISTRIBUTION IN AGING RATS

A Dissertation

by

MICHAEL WIECHMANN RAMSEY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2005

Major Subject: Kinesiology

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Approved by:

Chair of Committee, Michael Delp
Committee Members, Robert Armstrong
Judy Muller-Delp
Janet Parker
Head of Department, Steve M. Dorman

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ABSTRACT

Effects of Head-up Tilt on Mean Arterial Pressure, Heart Rate, and Regional Cardiac Output Distribution in Aging Rats. (December 2005)

Michael Wiechmann Ramsey, B.S.; M.A.,

Sam Houston State University

Chair of Advisory Committee: Dr. Michael Delp

Many senescent individuals demonstrate an inability to regulate mean arterial pressure (MAP) in response to standing or head-up tilt; however, whether this aging effect is the result of depressed cardiac function or an inability to reduce peripheral vascular conductance remains unknown. Therefore, the purpose of this research was to investigate the effects of aging on MAP, heart rate (HR), regional blood flow (via radioactive-microspheres), and vascular conductance during head-up tilt in conscious young (4 mo; n=12) and old (24 mo; n=10) male Fischer-344 rats. Heart rate and MAP were measured continuously during normal posture and during 10 minutes of head-up tilt. Blood flow was determined during normal posture and at the end of 10 minutes of head-up tilt. Young rats increased MAP significantly at the onset of head-up tilt and generally maintained the increase in MAP for the duration of head-up tilt, while aged rats showed a significant reduction in MAP after 10 minutes of head-up tilt. In the normal posture, aged rats demonstrated lower blood flow to splanchnic, bone, renal, and skin tissues versus young rats. With tilt there were decreases in blood flow to skin,

bone, and hind-limb in both age groups and in fat, splanchnic, reproductive, and renal tissues in the young. Bone blood flow was attenuated with age across both conditions in hind foot, distal femur, femur marrow, and proximal and distal tibia. Head-up tilt caused a decrease in blood flow across both age groups in all bones sampled with the exception of the hind foot. These results provide evidence that the initial maintenance of MAP in aged rats during head-up tilt occurs through decreased regional blood flow and vascular conductance, and that the fall in pressure is not attributable to an increase in tissue blood flow and vascular conductance. Therefore, reductions in arterial pressure during head-up tilt are likely a result of an old age-induced reduction in cardiac performance. In addition, this is the first study to demonstrate a decreased bone vascular conductance in both young and old rats during head-up tilt.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

According to the United States Census Bureau, the elderly population in America is growing at a disproportionate rate compared to those below 60 years of age. For example, in the year 2000 there were an estimated 35 million Americans age 65 years or older, which is a ten-fold increase since 1900. While the current population aged 65 years or older represents about 13% of the total population, according to estimations, by 2030 one in five Americans will be at least 65 years of age. Currently individuals age 85 years and older represent the fastest growing segment of the population. This growth in the elderly population needs to be reflected in a greater importance placed on research investigating problems associated with advanced age like cardiovascular disease, reduced functional capacity, and orthostatic hypotension.

Orthostatic Hypotension

Postural changes from supine or seated to upright position elicit an increase in the hemodynamic pressure gradient resulting in a blood volume shift from the thoracic cavity to the lower limbs (86). Moreover, this fluid shift, which results in reduced venous return, leads to a decrease in stroke volume and cardiac output with a corresponding increase in both heart rate and total peripheral resistance to maintain arterial blood pressure and cerebral blood flow (86). With advancing age, the change in posture from supine to standing results in a greater occurrence of orthostatic hypotension (OH) (42, 63, 90, 103). Orthostatic hypotension in humans has been defined as a

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decrease in systolic pressure of ≥ 20mmHg and/or a decrease in diastolic pressure of ≥ 10mmHg (103). There are a variety of hemodynamic mechanisms that could be responsible for this decreased tolerance of the upright posture in the elderly. An attenuation in one or more of these mechanisms may result in an inability to regulate regional blood flow and vascular conductance, thus reducing cerebral arterial pressure in aged individuals.

The detrimental effects of remaining in an upright posture for a continuous period of time have been known since man's early history. For example, ancient societies used crucifixion which entailed prolonged exposure to upright posture without the benefits of using the legs for support as a means of a slow and painful execution (86). While the ancients knew that this treatment would lead to death, the exact cause was not known for some time. Initially the cause of death was thought to be heart failure and/or asphyxiation. It is now known that death was a result of hypotension and cerebral ischemia, but this took many years of research in human and animal physiology to derive these conclusions. For example, Sir William Harvey (1578-1657), who is considered the father of the scientific method, was the first person to report on the circulation of blood and deduced the connection of the arteries to veins (104). Stephen Hales (1677-1761), the English clergyman who invented the manometer, was the first to accurately record blood pressure under a variety of physiological conditions. He also reported the importance of venous return on cardiac output (104). Hales work was followed by Dutch mathematician and physician Daniel Bernoulli (1700-1782), who was the first to make approximately correct calculations of cardiac output (104). Other important work

was done by Jean Leonard Marie Poiseuille (1799-1869) and Pierre LaPlace (1749-1827). Poiseuille was a pioneer in the area of the pressure/flow relationship, and LaPlace described the influence of transmural pressure on vessel diameter (104). These individuals laid the ground work for more recent discoveries in cardiovascular physiology that are the basis of much of our knowledge today.

The effect of posture on the circulation has been studied quite extensively in the young since the early 1900 (9, 37, 100, 111), but not until fairly recently in the elderly. While early studies have had mixed results (56), the preponderance of evidence is that the occurrence of orthostatic hypotension increases with advancing age. Findings reported from the Cardiovascular Health Study (87), a study of 5,201 men and women aged 65 years and older, were that 16.2% of the subjects became orthostatically hypotensive during a 3-minute stand test. The prevalence of orthostatic hypotension increased to 18.2% when those who could not finish the test due to dizziness upon standing were included in the results. Tilvis et al. (103), in a four-year follow-up to the Helsinki Aging Study, which looked at the frequency of orthostatic hypotension and/or dizziness in 569 individuals ages 75, 80, and 85 years old, reported that 30% of the subjects became orthostatically hypotensive during one minute of quiet standing. When data from only healthy subjects were considered, the occurrence of orthostatic hypotension was still at 26.6%. Subjects were identified as healthy if they were free from factors such as hypertension, diabetes, dementia, or symptoms of cardiovascular or pulmonary diseases, cancer or other disabling diseases, and had a normal exercise tolerance. Luukinen et al. (59) studied a population 792 individuals and found that 30%

of the subjects became orthostatically hypotensive at either 1 or 3 minutes of quiet standing. In another study of 319 elderly veterans, Myers (76) found that the prevalence of orthostatic hypotension in his study was only 4% in subjects during a 2-minute stand test. However the criterion used to define orthostatic hypotension in this study was a drop in mean arterial pressure of ≥ 20 mmHg as opposed to the ≥ 20 mmHg decline in systolic pressure or a ≥ 10 mmHg decline in diastolic pressure. The previous study not withstanding, the occurrence of orthostatic hypotension in the elderly as shown in large population studies has been shown to be anywhere from 16.2% to 31% (5, 11, 59, 87, 103).

While studies of large populations such as those listed above demonstrate the problem of orthostatic hypotension in the elderly, findings from research with smaller sample sizes have been more inconsistent. For example, in a study by Shi et al. (90) on 10 young and 10 aged individuals subjected to a lower body negative pressure (LBNP) of -40 Torr for 10 minutes the investigators found that on average the elderly subjects experienced a significant drop in systolic blood pressure compared to baseline while the young subjects demonstrated no such decrease. This difference was attributed to an increase in heart rate found in the young subjects. In contrast to this study, Minson et al. (67) found that the older subjects tolerated head-up tilt better than young subjects because of the older subject's greater ability to increase splanchnic vascular resistance. Furthermore, Lee et al. (56) found no differences between young and old subjects in response to 1 hour of 45° head-up tilt, which relates to about 70% of the hydrostatic

pressure gradient of standing, as opposed to 70° head-up tilt, which is equal to 94% of the hydrostatic pressure gradient of standing (94).

The varying results in the above mentioned studies suggest that research in the area of age and orthostatic hypotension is greatly influenced by differences in experimental approach. For example, many studies use different criteria for evaluating orthostatic intolerance. The most accepted criteria now is a decrease in systolic pressure of ≥ 20mmHg and/or a decrease in diastolic pressure of ≥ 10mmHg but earlier studies have used many different criteria.

Methods of Testing Orthostatic Intolerance

The most common methods of testing hemodynamic responses to an orthostatic challenge are active standing, head-up tilt and lower body negative pressure. Although the results from head-up tilt and active standing are quite similar, there are reports of differences between the two in elderly subjects. For instance, Imholz et al. (39) demonstrated that during active standing there was an initial rise followed by an abrupt fall in both systolic and diastolic pressures that was not seen during head-up tilt. Although research is lacking, it is likely that the skeletal muscle pump would be less active during head-up tilt than in active standing due to the more relaxed position.

In comparing lower body negative pressure and head-up tilt, while these two methods are similar in that they both elicit decreases in thoracic blood volume and central venous pressure, decreases in stroke volume and cardiac output, increases in heart rate and total peripheral resistance, decreases in pulse pressure, blood pooling in the lower extremities, hyperventilation, and finally syncope (95), they also have their

differences. There are physical differences in the direction and nature of the hydrostatic pressure change and there is a gravitational redistribution of pulmonary blood flow with head-up tilt (95). The downward displacement of abdominal organs and diaphragm is greater in head-up tilt. Additionally, with lower body negative pressure there are lesser decreases in cardiac output (20 to 40%) and greater increases in total peripheral resistance (40 to 100%) in older vs. younger subjects than are found during head-up tilt (95). Finally, with lower body negative pressure there is no activation of the vestibulo-sympathetic reflex, which affects sympathetic nerve activation and, consequently, vascular resistance (70). While lower body negative pressure has advantages for use in bed rest and microgravity studies, it could be argued that head-up tilt would be superior in many aspects relative to the other methodologies for examining orthostatic tolerance.

The use of head-up tilt with quadrapedal animal models is not a new approach. In dogs, DiBona and colleagues (22) showed reduced sodium absorption and increased renin release in anesthetized dogs subjected to 60° head-up tilt. Peterson et al. (80) investigated renal responses to 45° head-up tilt in anesthetized dogs. They found urine flow as well as urinary sodium excretion to be diminished with tilt. These results were abolished when neural input to the kidneys was removed. Furthermore Bedford and Dormer (6) studied arterial hemodynamic responses to head-up tilt in conscious dogs. They found no change in heart rate, an increase in mean arterial pressure, reduced stroke volume and cardiac output, and a diminished blood flow to the kidneys. Rabbits were subjected to 70° head-up tilt in a study by Singh et al. (92), which resulted in increases in mean arterial pressure and heart rate from baseline measurements. In rats, Golin and

colleagues found that renin (30) and arginine vasopressin (31) were increased during 45° head-up tilt and that propranolol caused reductions in mean arterial pressure and heart rate with tilt (31).

Cardiac Output Distribution

Past research has demonstrated that there is altered distribution of cardiac output with senescence both at rest and when various stresses are placed on subjects (16). Minson and colleagues (67) subjected young and elderly humans to 70° head-up tilt and measured the postural cardiovascular responses. Elderly subjects had an augmented vascular resistance and decreased blood flow to the splanchnic region concomitant with an attenuated ability to reduce forearm blood flow. In a more recent study by Delp et al. (19), it was reported that the percent of cardiac output going to adipose tissue at rest increased with age, whereas the percent of cardiac output to skin and reproductive tissues was decreased. In addition, a study by Musch and colleagues (75) showed a decrease in blood flow to the kidneys, spleen, and stomach in aged rats compared to young rats at rest. Castellano et al. (12) measured blood flow in the carotid and femoral arteries using Doppler ultrasound in 37 men and women ages 69 to 82 years old. They reported a 17% decrease in carotid blood flow and a 60% decrease in femoral blood flow immediately after head-up tilt. From these studies it is evident that regional vascular regulation is affected by old age both at rest and when the body is subjected to tilt.

Another stressor that has been used to test cardiovascular function is exercise. Research using animal models during exercise has shown that there is altered cardiac output distribution with old age. Tuma et al. (105) used radio-labeled microspheres in

Fischer 344 virgin female rats and found that blood flow to the brain and kidneys was reduced in senescent rats. Haidet (35) found the reduction in splanchnic blood flow to be significantly greater in aged maximally exercising beagles when compared to young control animals. In a study by Irion and coworkers (40), blood flow to electrically stimulated skeletal muscles in aged rats was found to be significantly lower than corresponding flows in young adult rats. The results from the exercise condition of the study by Musch and colleagues (75) demonstrated that while blood flow to the total hind limb was not significantly different, there was a redistribution of blood flow from highly oxidative muscles to highly glycolytic muscles. Blood flow response to exercise in active skeletal muscle appears to be reduced with age in humans. For example, Ho et al. (38) found in fit aged men that less blood flow was shunted away from their splanchnic and kidney circulations than in younger control subjects during exercise. Wahren et al. (108) reported that knee extensor blood flow and oxygen consumption during upright leg cycling were lower in men with a mean age of 54 compared with exercise responses reported in an earlier investigation of younger men (45). In a recent study by Proctor et al. (82), leg blood flow and vascular conductance during knee extension exercise was significantly lower in older endurance trained men when compared to their younger counter parts. Thus, there are obvious differences between the young and aged regarding blood flow distribution during the stress of exercise as well as orthostatic stress.

Myogenic Response

Changes in the vasomotor properties of the resistance vasculature may underlie some of the alterations in blood flow distribution that occurs with old age. For example, there is recent evidence that aged rats have a diminished myogenic response (32, 57, 73). The myogenic response is a key autoregulatory feature of the microvasculature that aids in the maintenance of arterial pressure and tissue blood flow by responding to changes in transmural pressure. When there is an increase in transmural pressure the vessel constricts, or conversly when there is a decrease in transmural pressure the vessel dilates to maintain normal tissue blood flow. An attenuated myogenic response could play a key role in elevating peripheral vascular conductance and, consequently, increasing the occurrence of orthostatic hypotension during an increase in the hydrostatic pressure gradient induced by a change to the upright posture. Muller-Delp et al (73) isolated arterioles from the gastrocnemius and soleus muscles from young (4 mos) and aged (26 mos) Fischer 344 rats. They found that the percent constriction due to an increase in transmural pressure (myogenic response) was significantly reduced in aged rats when compared to young rats. Research by Gros et al. (32) found that the myogenic response set point, the pressure at which there is a significant vasoconstriction, increases with age in mesenteric arteries of mice. This corresponded with an increase in blood pressure with age. A more recent study by Lott et al. (57) showed a decreased dynamic vasoconstriction in aged men and women to alterations in forearm transmural pressure while steady-state constriction was enhanced. Attenuation of the myogenic activity and

vascular responsiveness in the hind-limb vasculature could lead to increased vascular conductance that could possibly diminish mean arterial pressure and cerebral blood flow.

Baroreceptor Function

The mechanoreceptors of the baroreflexes sense changes in pressure within the circulatory system and adjust cardiovascular control mechanisms to maintain homeostasis (86). There is evidence in the literature that the baroreflex systems in the aged have an attenuated ability to defend against changes in pressure. For example, in a study by Wei et al. (109), which used head-up tilt and changes in blood volume to study baroreflex activity in young (6 mos.) and senescent (24 mos.) Fischer 344 rats, they found that the heart rate response to both increases and decreases in pressure was diminished in the senescent rats. Tanabe and Bunag (101) showed baroreflex responses to various stimuli were impaired in aged female Sprauge-Dawley rats when compared to their younger counterparts. The aged rats had a diminished heart rate and sympathetic nerve response to phenylephrine, sodium nitroprusside, and electrical stimulation of the left aortic depressor nerve. The most notable deficits to the baroreflexes occur with the sympathetic branch of the autonomic nervous system affecting the heart, while control of the vascular beds appears to be less affected with age in rats than in humans. This is thought to be due to the greater extent of arterial wall stiffening that occurs in humans as opposed to rats.

Heart Rate Response

Increases in heart rate reflect a key response in maintaining cardiac output as well as cerebral blood flow. There is evidence that the increase in heart rate triggered by

orthostatic stress is attenuated with age and that this may be a direct cause of orthostatic hypotension in the elderly (90). Jansen and colleagues (42) showed a blunted increase in heart rate in elderly men subjected to 45° head-up tilt. Similar findings were also shown in a tilt study by Smith et al. (94), who used 70° head-up tilt in young (20 to 29 years), middle-aged (40 to 49 years) and older (60 to 69 years) healthy men, the older group showed a significantly attenuated heart rate response during tilt when compared to the other two age groups. In the aged heart there is a prolonged duration of contraction with a delayed relaxation that aides in the maintenance of stroke volume but limits maximal heart rate (53). There is also an altered responsiveness to β-adrenergic stimuli in the heart. This can attenuate the cardioacceleration response to various stresses in the aged individual (53).

Vascular Responsiveness

Vascular responsiveness to chemical stimuli also seems to be diminished with age. For example, Cook et al. (15) found a significant decrease in the cremaster muscle microvascular responsiveness to adenosine in aged rats. In the same study they found no difference in response to norepinephrine between young and aged rats. Similar results have been shown regarding vascular responsiveness to norepinephrine in both isolated rat skeletal muscle arterioles (73) and human forearm cutaneous circulation (112). Studies from various vascular beds have shown that endothelium-dependant vasodilation is diminished in the aged. In particular, Delp et al. (17) found that acetylcholine-induced vasodilation was impaired by aging in the rat aorta, and Koga et al. (51) had similar results in rat mesenteric arteries. In skeletal muscle arterioles, Muller-Delp et al. (74),

as well as Spier et al. (96), reported reduced vasodilation in response to acetylcholine in first-order arterioles isolated from the soleus muscle of aged Fisher 344 rats when compared to young rats. There is evidence that endothelial-dependent vasoconstriction is enhanced with age. The vasoconstrictor response to endothelin, an endothelial-derived vasoconstrictor agent, has been shown to be enhanced with age in arterioles isolated from the white portion of the gastrocnemius muscle in Fisher 344 rats (27). This enhancement is thought to be due to an increased sensitivity of the ET_A receptor found in smooth muscle, while the ET_B receptor located on the smooth muscle and endothelial cells showed no age-induced enhancement in sensitivity.

Sympathetic Stimulation

The role of the sympathetic nervous system is critical to the maintenance of arterial blood pressure during resting conditions and while the body is under physiological stress. While aging is associated with an increase in sympathetic nervous system activity, there are differences in the response of various tissues within the body. The heart becomes less responsive to sympathetic stimulation as a person ages (53), which can limit the capacity of the heart to meet the demands of various stresses, such as exercise or an orthostatic challenge. This is thought to be due to the attenuated α_2 mediated response at the pre-synaptic as well as post-synaptic sites. The attenuation can be seen in the reduced negative-feedback on the junctional release of norepinephrine as well as the reduced effectiveness of the reuptake mechanism. According to Folkow and Svanborg (29) the elevated plasma norepinephrine concentrations reflect a combination of 1) an increase in sympathetic discharge, 2) an increase neurotransmitter release per

impulse, 3) reduced norepinephrine reuptake at the nerve junctions, and 4) reduced elimination by the liver and kidneys. This increase in plasma norepinephrine might be a compensatory mechanism for the declining efficiency of cardiac and vascular smooth muscle function.

Vascular resistance is an integral element in the regulation of blood flows to different tissues, as well as the maintenance of arterial blood pressure (21). There is evidence that the elderly compensate for decreased cardioacceleration during an orthostatic event with an increase in vascular resistance to the splanchnic tissues of the body (67, 91). This increase in vascular resistance to the splanchnic region helps the elderly maintain stroke volume and thereby maintain cardiac output without an increase in heart rate. Interestingly, the elderly maintain their stroke volume even though there is a significant increase in blood flow to the forearm during head-up tilt due to an attenuated ability to increase forearm vascular resistance (67). Although the aforementioned research suggests an augmented vascular resistance in the splanchnic region with age, which would seem to aid orthostatic tolerance, there is still an increased incidence of orthostatic hypotension with age. There is evidence of decreased basal whole-limb (forearm) vascular conductance with age (23, 24), which is thought to be mediated through chronically elevated sympathetic vasoconstriction (25). At this time there is no comprehensive study identifying specific tissues in the body where vascular resistance might be altered with age during head-up tilt.

Presently, no data are available in the literature to document the effects of aging on arterial pressure, heart rate, and cardiac output distribution during head-up tilt in the

rat model. Although published studies with humans have addressed the issue of altered perfusion of select visceral, subcutaneous, and skeletal muscle tissues during head-up tilt with aging (67, 91), the present study will provide a more comprehensive analysis of the effects of aging on regional blood flows and vascular conductance concomitant with measurements of mean arterial pressure and heart rate during head-up tilt among the various organ systems. Therefore, the purpose of the present study was to test the hypothesis that there is a diminished ability to maintain MAP during orthostasis in aged Fischer-344 rats, and to identify whether perfusion and vascular dysfunction in specific tissues may be responsible for this attenuation. If the results indicate the aged animals exhibit an attenuated ability to lower vascular conductance to more compliant tissues or tissues located in the periphery, this could provide insight into a potential mechanism responsible for an increased incidence of orthostatic intolerance among the elderly.

METHODS

Animals

Young adult (4 month old) and aged (24 month old) male Fischer 344 rats were obtained (National Institute of Aging colony). These ages were chosen to correspond to the normal life span and sexual development of the Fischer-344 rats, e.g., 3- to 6-mo-old rats represent young sexually mature adult animals and ≥ 24-mo-old rats are considered senescent (26). The rats were housed in a temperature-controlled (23±2°C) room with a 12:12-h light dark cycle. Water and rat chow were provided ad libitum. Prior to the surgical procedure, animals were habituated to the tilt apparatus at 0˚ tilt for 15 min per day for at least 5 days.

Surgical Procedures

At the conclusion of the habituation regimen, the rats were anesthetized with isoflurane (2%) and a catheter (Dow Corning, Silastic; ID 0.6 mm, OD 1.0 mm) filled with heparinized (Elkins-Sinn Inc., 100 U/mL) saline solution was advanced towards the left ventricle via the right carotid artery as previously described (20). This catheter was used subsequently for infusion of radiolabelled microspheres for tissue blood flow measurements and for the recording of arterial pressures. The carotid catheter was externalized at the base of the tail and secured on the underside of the tail and the incision was closed with suture (3-0 silk, Davis+Geck, Puerto Rico). A second polyurethane catheter (Braintree Scientific; ID 0.36 mm; OD 0.84 mm) filled with heparinized saline solution was implanted in the caudal tail artery and externalized at the

tail as described previously (16). This catheter was used to obtain a reference blood sample, which serves as an artificial organ for calculating tissue flows.

Experimental Protocol

After a 24 h recovery period the animals were placed in the tilt apparatus in the horizontal position (0° tilt). The rats were allowed 20 min to stabilize before the first microsphere infusion. Arterial pressure and heart rate were measured in each rat at 0˚ tilt for baseline data and every minute up to 10 min immediately after the onset of tilt at 70˚. Tissue blood flows (radiolabelled microsphere technique) were measured during 0˚ tilt and after 10 min of 70˚ tilt. At the end of the experiment, the animals were euthanized with an overdose of sodium penobarbital (>80 mg/kg, i.a.). Visceral tissues (kidneys, spleen, stomach, duodenum, large intestine, adrenal glands, liver), hind-limb skin, fat (subcutaneous, abdominal, epididymal), bone (femur, patella, tibia, fibula, hind-foot, tail), and 19 hind-limb muscle samples were excised, weighed, and placed into counting vials for blood flow determination and calculation of tissue vascular conductance.

Simulated Orthostatism.

Animals were gently restrained in a Plexiglas canopy (Rodent ECU, Braintree Scientific) hinged to a tilting Plexiglas support base. The animals were placed so that the thorax is at the same level as the tilting axis. The design for the tilting apparatus is based on the experimental model developed by Martel et al. (62). The head end of the canopy had a tapered opaque plastic hood to protect the rodents from visual disturbances. This served to minimize the ocular postural input, which may alter

cardiovascular reflex responses (16). Tilt time was ~1.5 s from level (0°) baseline condition to 70° head-up tilt.

Blood Flow and Vascular Conductance Measurements

Radiolabelled (^{46}Sc, and ^{85}Sr) microspheres (Perkin Elmer NEN) with a 15.5 ± 0.2-μm diameter were used for blood flow measurements, as described previously (16). Specifically, microspheres were suspended in physiological saline with <0.5% Tween 80 and mixed prior to infusion by 10 min sonication (FS20 Sonicator, Fisher Scientific) followed by 1 min of agitation on a vortex mixer (Fisher Scientific). A reference blood sample was taken from the caudal artery at a rate of 0.618 ml/min with an infusion/withdrawal pump (Harvard). Ten seconds later, ~0.5 million spheres suspended in 0.2 ml saline were infused into the carotid catheter over a 10- to 15-s period. One ml of warm (37°C) saline was infused over a 30-s period immediately after microsphere infusion; withdrawal of the reference blood sample continued for at least one min after the saline flush. After euthanasia and tissue dissection, tissue samples were counted in a gamma counter (Packard Auto-Gamma 5780), and flows were computed (IBM PC computer) from counts per min and tissue wet weights (blood flows reported in ml · min^{-1} · 100 g^{-1}). Microsphere mixing was assessed by comparing bilateral kidney flows, and were considered adequate if bilateral flows were within 15% of each other. Pressure recordings, made with pressure transducers (Electromedical) and recorded on a data acquisition system (MacLab/Macintosh), were made immediately before and after the microsphere infusion and averaged, since simultaneous pressure measurements, blood withdrawal and microsphere infusion were not possible. Mean arterial pressure was

electronically averaged from pulsatile pressure measurements from the carotid catheter. Heart rate was estimated from pulsatile pressure tracings from the carotid catheter. Regional vascular conductances (ml · min^{-1} · $100g^{-1}$ · $mmHg^{-1}$) were calculated by dividing tissue flows (ml · min^{-1} · 100 g^{-1}) by the mean arterial pressure (mmHg).

Baroreceptor Function

The rats were anesthetized with isoflurane (2%) and a catheter (Dow Corning, Silastic; ID 0.6 mm, OD 1.0 mm) filled with heparinized (Elkins-Sinn Inc., 100 U/mL) saline solution was advanced towards the left ventricle via the right carotid artery as previously described (20). This catheter was used subsequently for the recording of heart rate and arterial pressures. A silastic catheter (Dow Corning; ID 0.6mm, OD 1.0mm) was advanced towards the right atria via the right jugular vein and externalized. The carotid catheter was externalized to the dorsal cervical region and the incision was closed with suture (3-0 silk, Davis+Geck, Puerto Rico). Precautions were taken to minimize disturbances due to noise, lighting, or the movements of personnel in the laboratory, which can acutely affect blood pressure and heart rate. Rats were placed in a cage which the sides had been covered to mask the presence and movements of laboratory personnel.

Hypotension was induced using a bolus injection of sodium nitroprusside (Sigma) via the venous cannula. Nitroprusside concentration was adjusted to give a final dose of 50 μg/kg. A ΔMAP was calculated by subtracting the lowest measured mean arterial pressure from the baseline mean arterial pressure. The ΔMAP was then divided by the time it took for MAP to return to baseline levels from lowest measured

point. This calculated number was used as a relative rate constant compared between the two groups for statistical significance.

Statistical Analysis

A two-way, repeated measures ANOVA was used to compare mean arterial pressures, heart rates, tissue blood flows, and vascular conductance between groups (young adult vs. senescent) and within groups across conditions (rest vs. 75˚ tilt). Duncan's multiple range test was used to determine the significance of difference among treatment means. A $P<0.05$ was used to indicate significance.

RESULTS

Animals

Body mass was significantly greater with old age. The mean weight of the young rats was 341 ± 7 g while the mean weight of the aged rats was 405 ± 7 g.

Heart Rate and Mean Arterial Pressure

Heart rate was significantly lower in the aged rats during the last two time points of head-up tilt compared to baseline values (Fig. 1). Heart rate values in the young rats remained above baseline values throughout tilt but only significantly at the 2 minute time point. Heart rate values did not differ between the two groups until the last two time points when the heart rate response in the aged rats was significantly lower than that of the young rats. Mean arterial pressure in the aged rats rose slightly upon acute head-up tilt and remained stable until the 4-min time point after which pressure fell steadily with a significant decrease from baseline at the 10-min time point. In the young rats, mean arterial pressure was significantly elevated during acute head-up tilt and with the exception of the 6-min and 8-min time points remained significantly above baseline (Fig. 2). There were no significant differences in baroreceptor function between the young and aged animals.

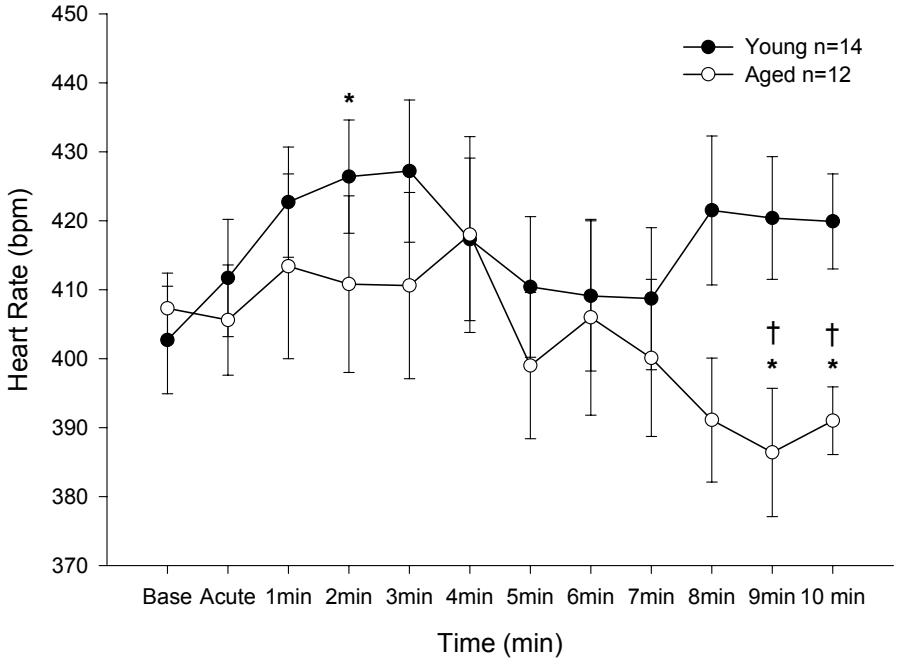

Fig. 1. Heart Rate. Values are means ± SE. † Aged mean is different from corresponding young mean, *P<0.05*. * Time point mean is different from corresponding baseline mean, *P<0.05*.

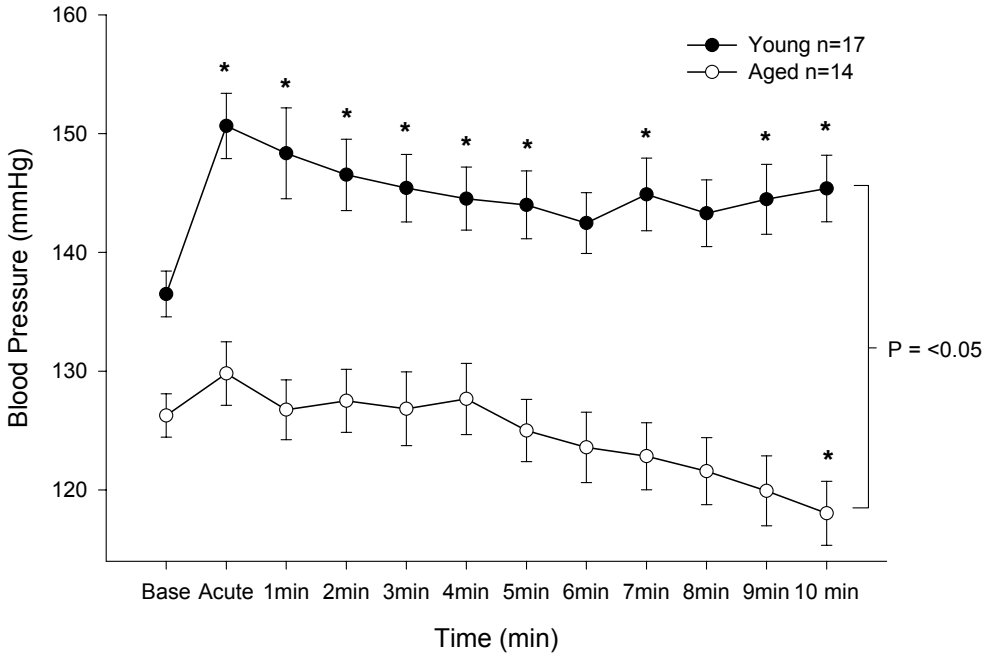

Fig. 2. Blood Pressure. Values are means ± SE. * Time point mean is different from corresponding baseline mean, *P<0.05*.

Group Tissue Blood Flows

Regional blood flows were lower with age in the splanchnic, skin, bone and renal tissues across both baseline and 10-min head-up tilt conditions. There was a reduction in regional blood flows after 10 min of head-up tilt in skin, bone, and hind-limb in both young and aged rats. While a reduction regional blood flows after 10 min of head-up tilt was seen only in the young rats in fat, splanchnic, reproductive tissue, and renal tissues (Table 1).

Group Tissue Vascular Conductance

Regional vascular conductance was significantly reduced with 10 min of head-up tilt to splanchnic, skin, bone, and hind-limb tissues in both young and aged rats. Only young rats reduced regional vascular conductance after 10 min of head-up tilt in fat, reproductive, and renal tissues (Table 2).

Table 1. *Group tissue blood flow*

	Baseline		Tilt	
	Young	Aged	Young	Aged
Fat	15±1	13±2	9±1 *	12±2
Splanchnic	216±26	128±13 †	173±22 *	112±14 †
Muscle	32±7	32±5	28±6	25±4
Skin	8±1	5±1 †	5±1 *	4±1 †*
Bone	17±2	12±1 †	9±1 *	5±1 †*
Repo/Other	23±2	20±2	18±2 *	18±2
Hind Limb	27±5	25±3	21±4 *	17±3 *
Renal	551±33	338±36 †	445±52 *	308±34 †

Values are means ± SE in ml • min^{-1} • 100 g^{-1}. † Aged mean is different from corresponding young mean, $P<0.05$. * 10 minute head-up tilt mean is different from corresponding baseline mean, $P<0.05$.

Table 2. *Group tissue vascular conductance*

	Baseline		Tilt	
	Young	Aged	Young	Aged
Fat	0.106±0.008	0.113±0.017	0.065±0.009 *	0.107±0.023 †
Splanchnic	1.55±0.171	1.11±0.127	1.2±0.155 *	1.03±0.14
Muscle	0.228±0.051	0.292±0.058	0.189±0.042	0.221±0.034
Skin	0.056±0.005	0.044±0.004	0.035±0.005 *	0.033±0.005 *
Bone	0.125±0.011	0.104±0.009	0.062±0.007 *	0.049±0.006 *
Repo/Other	0.168±0.014	0.176±0.021	0.128±0.017 *	0.163±0.02
Hind Limb	0.198±0.036	0.226±0.04	0.143±0.028 *	0.153±0.02 *
Renal	3.97±0.21	2.9±0.28	3.1±0.37 *	2.8±0.32

Values are means ± SE in ml • min^{-1} • 100 g^{-1} • $mmHg^{-1}$. † Aged mean is different from corresponding young mean, $P<0.05$. * 10 minute head-up tilt mean is different from corresponding baseline mean, $P<0.05$.

Individual Tissue Blood Flows

Individual tissue flows were lower in the aged rats when compared to the young rats across both baseline and 10-min of head-up tilt in spleen, mesentery, large intestine and rectum, prostate, adrenal cortex, left kidneys, and urinary bladder. The aged rats showed attenuated blood flow compared to young rats in subcutaneous fat and right kidney only at baseline. In the young animals the 10 minute tilt condition elicited a reduction in blood flow in subcutaneous fat, epididymal fat, spleen, mesentery, cecum, testis, and left and right kidneys. Blood flows were reduced at 10 minutes of head-up tilt in both young and aged rats in stomach, hind-limb skin, and tail skin (Table 3).

Individual Tissue Vascular Conductance

Individual tissue vascular conductance was diminished with age across both conditions in spleen, left kidney, and urinary bladder. Increases in individual vascular conductance with age in both conditions could be seen in abdominal fat, testis, and adrenal medulla. Reductions in individual tissue vascular conductance with tilt in both age groups were seen in the stomach, mesentery, large intestine and rectum, seminal vesicles, testis, and tail skin. Ten minutes of head-up tilt resulted in diminished vascular conductance only in the young rats in subcutaneous fat, epididymal fat, spleen, pancreas, cecum, hind-limb skin, and left and right kidneys (Table 4).

Table 3. *Tissue blood flows from young and aged rats*

	Baseline		Tilt	
Tissue	Young	Aged	Young	Aged
Adipose				
Abdominal fat	17 ± 2	20 ± 3	12 ± 2	19 ± 4
Subcutaneous fat	19 ± 2	11 ± 2†	10 ± 2*	8 ± 2
Epididymal fat	9 ± 1	9 ± 2	6 ± 1*	8 ± 2
Splanchnic				
Spleen	199 ± 20	53 ± 12†	138 ± 24*	42 ± 11†
Stomach	165 ± 22	114 ± 9	115 ± 18*	79 ± 8*
Duodenum	346 ± 44	234 ± 24	311 ± 46	212 ± 26
Jejunem and ileum	270 ± 40	197 ± 36	242 ± 36	206 ± 42
Pancreas	298 ± 70	151 ± 42	228 ± 47	132 ± 41
Mesent	31 ± 4	19 ± 3†	25 ± 3*	15 ± 4†
Cecum	233 ± 30	145 ± 25	172 ± 27*	123 ± 21
Large intestine and rectum	183 ± 27	108 ± 25†	151 ± 25	86 ± 22†
Repoductive				
Seminal vesicles	12 ± 2	10 ± 2	8 ± 1	8 ± 2
Prostate	27 ± 4	14 ± 2†	22 ± 4	13 ± 2†
Testis	31 ± 1	36 ± 5	25 ± 2*	33 ± 4
Skin				
Hindlimb skin	13 ± 2	8 ± 1	8 ± 1*	6 ± 1*
Tail skin	3 ± 0.4	2 ± 1	2 ± 0.3*	2 ± 0.3*
Other				
Adrenal Medulla	109 ± 11	123 ± 14	114 ± 13	137 ± 18
Adrenal Cortex	906 ± 123	510 ± 74†	936 ± 119	492 ± 69†
Left Kidney	573 ± 38	334 ± 35†	461 ± 56*	294 ± 31†
Right Kidney	530 ± 34	344 ± 39†	430 ± 48*	323 ± 38
Urinary Bladder	33 ± 4	18 ± 3†	26 ± 3	16 ± 1†
Liver	8 ± 3	10 ± 6	7 ± 2	8 ± 4

Values are means ± SE in ml • min^{-1} • 100 g^{-1}. † Aged mean is different from corresponding young mean, $P<0.05$. * 10 minute head-up tilt mean is different from corresponding baseline mean, $P<0.05$.

Table 4. *Tissue vascular conductance from young and aged rats*

	Baseline		Tilt	
Tissue	Young	Aged	Young	Aged
Adipose				
Abdominal fat	0.118 ± 0.011	0.178 ± 0.03†	0.0866 ± 0.012	0.172 ± 0.04†
Subcutaneous fat	0.135 ± 0.016	0.089 ± 0.013†	0.0689 ± 0.013*	0.078 ± 0.017
Epididymal fat	0.065 ± 0.004	0.0731 ± 0.014	0.0405 ±0.006*	0.0709 ± 0.016
Splanchnic				
Spleen	1.431 ± 0.135	0.443 ± 0.098†	0.959 ± 0.164*	0.381 ± 0.09†
Stomach	1.179 ± 0.149	1.023 ± 0.13	0.801 ± 0.128*	0.721 ± 0.08*
Duodenum	2.496 ± 0.315	2.034 ± 0.23	2.176 ± 0.343	1.94 ± 0.258
Jejunem and ileum	1.925 ± 0.261	1.723 ± 0.316	1.679 ± 0.246	1.852 ± 0.353
Pancreas	2.147 ± 0.505	1.351 ± 0.38	1.576 ± 0.325*	1.29 ± 0.414
Mesent	0.225 ± 0.03	0.163 ± 0.032	0.177 ± 0.027*	0.136 ± 0.037*
Cecum	1.667 ± 0.2	1.239 ± 0.21	1.192 ± 0.186*	1.108 ± 0.177
Large intestine and rectum	1.311 ± 0.18	0.937 ± 0.213	1.038 ± 0.169*	0.777 ± 0.195*
Repoductive				
Seminal vesicles	0.0908 ± 0.017	0.0914 ± 0.017	0.0537 ± 0.007*	0.0765 ± 0.02*
Prostate	0.188 ± 0.03	0.124 ± 0.018	0.154 ± 0.031	0.119 ± 0.022
Testis	0.224 ± 0.01	0.314 ± 0.041†	0.175 ± 0.017*	0.293 ± 0.038†*
Skin				
Hindlimb skin	0.093 ± 0.012	0.0679 ± 0.005	0.0552 ± 0.009*	0.0534 ± 0.008
Tail skin	0.0182 ± 0.003	0.0195 ± 0.004	0.0141 ± 0.002*	0.0134 ± 0.003*
Other				
Adrenal Medulla	0.796 ± 0.086	1.058 ± 0.113†	0.799 ± 0.1	1.226 ± 0.143†
Adrenal Cortex	6.56 ± 0.88	4.379 ± 0.593	6.582 ± 0.872	4.432 ± 0.581
Left Kidney	4.126 ± 0.25	2.857 ± 0.268†	3.198 ± 0.393*	2.678 ± 0.313†
Right Kidney	3.826 ±0.23	2.941 ± 0.298	2.999 ± 0.351*	2.923 ± 0.34
Urinary Bladder	0.24 ± 0.03	0.146 ± 0.015†	0.183 ± 0.023	0.146 ± 0.013†
Liver	0.0574 ± 0.022	0.0986 ± 0.063	0.0494 ± 0.017	0.0758 ± 0.041

Values are means ± SE in ml • min^{-1} • 100 g^{-1} • $mmHg^{-1}$. † Aged mean is different from corresponding young mean, *P<0.05*. * 10 minute head-up tilt mean is different from corresponding baseline mean, *P<0.05*.

Skeletal Muscle Blood Flow and Vascular Conductance

Skeletal muscle blood flow was diminished with 10 min of head-up tilt in the soleus muscle, which occurred in both young and aged animals (Table 5). There were increases in vascular conductance with age across both conditions in the adductor muscles and the white portion of the gastrocnemius muscle. The soleus muscle elicited a decrease in vascular conductance after 10 min of tilt in both age groups (Table 6).

Table 5. *Skeletal muscle blood flows from young and aged rats*

	Baseline		Tilt	
Tissue	Young	Aged	Young	Aged
Trunk and hip				
Superficial gluteal/tensor fasciae latae	19 ± 4	16 ± 2	17 ± 3	15 ± 3
Medial gluteal	21 ± 5	22 ± 3	24 ± 5	23 ± 4
Adductors	19 ± 4	24 ± 3	21 ± 4	24 ± 3
Thigh				
Knee Flexors	13 ± 3	14 ± 2	16 ± 4	14 ± 3
Vastus Intermed	48 ± 17	44 ± 12	43 ± 13	22 ± 3
Vastus Medialis	28 ± 7	29 ± 5	27 ± 7	30 ± 5
Rectus Femorus	36 ± 9	34 ± 7	32 ± 10	22 ± 5
Vastus lateralis, red	58 ± 16	54 ± 21	47 ± 13	30 ± 9
Vastus lateralis, mixed	33 ± 8	39 ± 10	35 ± 8	29 ± 6
Vastus lateralis, white	16 ± 3	13 ± 3	17 ± 4	12 ± 3
Leg				
Soleus	53 ± 14	57 ± 21	24 ± 7*	14 ± 5*
Plantaris	18 ± 4	24 ± 6	20 ± 4	19 ± 5
Gastrocnemius, red	20 ± 6	20 ± 3	25 ± 6	19 ± 6
Gastrocnemius, mixed	22 ± 5	27 ± 4	28 ± 7	23 ± 4
Gastrocnemius, white	16 ± 3	20 ± 4	16 ± 4	20 ± 4
Tibialis anterior, red	78 ± 21	50 ± 11	44 ± 13	53 ± 15
Tibialis anterior, white	36 ± 7	37 ± 6	28 ± 6	35 ± 6
Extensor digitorum longus	34 ± 8	50 ± 9	34 ± 11	41 ± 8
Other				
Cremaster	6 ± 2	9 ± 2	6 ± 1	6 ± 1
Diaphragm	68 ± 9	89 ± 18	68 ± 9	74 ± 16

Values are means ± SE in ml • min^{-1} • 100 g^{-1}. * 10 minute head-up tilt mean is different from corresponding baseline mean, $P<0.05$.

Table 6. *Skeletal muscle vascular conductance from young and aged rats*

	Baseline		Tilt	
Tissue	Young	Aged	Young	Aged
Trunk and Hip				
Superficial gluteal/tensor fasciae latae	0.137 ± 0.03	0.141 ± 0.02	0.119 ± 0.02	0.136 ± 0.03
Medial gluteal	0.152 ± 0.03	0.191 ± 0.03	0.167 ± 0.03	0.209 ± 0.03
Adductors	0.134 ± 0.03	0.216 ± 0.04†	0.143 ± 0.03	0.215 ± 0.03†
Thigh				
Knee Flexors	0.0946 ± 0.02	0.123 ± 0.02	0.112 ± 0.022	0.13 ± 0.03
Vastus Intermed	0.356 ± 0.14	0.421 ± 0.14	0.298 ± 0.1	0.195 ± 0.03
Vastus Medialis	0.208 ± 0.06	0.266 ± 0.06	0.184 ± 0.05	0.267 ± 0.04
Rectus Femorus	0.259 ± 0.07	0.323 ± 0.1	0.216 ± 0.06	0.203 ± 0.04
Vastus lateralis, red	0.422 ± 0.12	0.518 ± 0.23	0.321 ± 0.09	0.252 ± 0.08
Vastus lateralis, mixed	0.236 ± 0.06	0.37 ± 0.12	0.242 ± 0.06	0.254 ± 0.05
Vastus lateralis, white	0.116 ± 0.02	0.112 ± 0.03	0.119 ± 0.02	0.105 ± 0.03
Leg				
Soleus	0.387 ± 0.11	0.528 ± 0.21	0.161 ± 0.05*	0.126 ± 0.05*
Plantaris	0.13 ± 0.03	0.208 ± 0.05	0.134 ± 0.03	0.167 ± 0.04
Gastrocnemius, red	0.143 ± 0.04	0.179 ± 0.04	0.173 ± 0.04	0.168 ± 0.05
Gastrocnemius, mixed	0.161 ± 0.04	0.244 ± 0.05	0.192 ± 0.04	0.206 ± 0.03
Gastrocnemius, white	0.117 ± 0.02	0.184 ± 0.04†	0.107 ± 0.02	0.183 ± 0.03†
Tibialis anterior, red	0.558 ± 0.15	0.447 ± 0.10	0.302 ± 0.09	0.465 ± 0.13
Tibialis anterior, white	0.258 ± 0.05	0.33 ± 0.06	0.189 ± 0.04	0.316 ± 0.056
Extensor digitorum longus	0.241 ± 0.06	0.445 ± 0.09	0.23 ± 0.07	0.372 ± 0.08
Other				
Cremaster	0.0473 ± 0.013	0.0779 ± 0.018	0.0418 ± 0.007	0.0579 ± 0.011
Diaphragm	0.486 ± 0.058	0.829 ± 0.204	0.462 ± 0.057	0.667 ± 0.137

Values are means ± SE in ml • min^{-1} • 100 g^{-1} • $mmHg^{-1}$. † Aged mean is different from corresponding young mean, *P<0.05*. * 10 minute head-up tilt mean is different from corresponding baseline mean, *P<0.05*.

Bone Blood Flow and Vascular Conductance

Bone blood flow was attenuated with age across both conditions in hind foot, distal femur, femur marrow, proximal tibia, and distal tibia. Head-up tilt caused a significant decrease in blood flow across both age groups in all bones sampled with the exception of the hind foot (Table 7).

Vascular conductance in bone was reduced with age in only the femur marrow during both experimental conditions (Fig. 3). Meanwhile, all bone tissues sampled showed a decrease in vascular conductance with tilt across both age groups (Table 8).

Table 7. *Bone blood flows from young and aged rats*

	Baseline		Tilt	
Tissue	Young	Aged	Young	Aged
Tail	3 ± 1	4 ± 1	2 ± 0.4*	2 ± 1*
Patella, tendon, and ligament	19 ± 5	13 ± 2	7 ± 2*	2 ± 1*
Foot (hindlimb)	13 ± 2	8 ± 1†	12 ± 1	6 ± 1†
Femur-Proximal	26 ± 3	21 ± 2	17 ± 2*	11 ± 1*
Femur-Shaft	15 ± 2	13 ± 2	6 ± 1*	6 ± 1*
Femur-Distal	30 ± 4	21 ± 3†	17 ± 2*	11 ± 2†*
Femur-Marrow	39 ± 7	18 ± 5†	18 ± 4*	7 ± 2†*
Tibia-Proximal	29 ± 3	19 ± 2†	15 ± 2*	12 ± 1†*
Tibia-Shaft	9 ± 2	9 ± 1	2 ± 1*	2 ± 1*
Tibia-Distal	6 ± 2	3 ± 1†	3 ± 1*	0± 0†*
Fibula	3 ± 1	5 ± 2	0 ± 0*	0 ± 0*

Values are means ± SE in ml • min^{-1} • 100 g^{-1}. † Aged mean is different from corresponding young mean, $P<0.05$. * 10 minute head-up tilt mean is different from corresponding baseline mean, $P<0.05$.

Table 8. *Bone vascular conductance from young and aged rats*

	Baseline		Tilt	
Tissue	Young	Aged	Young	Aged
Tail	0.0191 ± 0.004	0.0315 ± 0.01	0.0122 ± 0.003*	0.0185 ± 0.01*
Patella, tendon, and ligament	0.141 ± 0.04	0.113 ± 0.02	0.0482 ± 0.01*	0.0145 ± 0.01*
Foot (hindlimb)	0.0934 ± 0.01	0.0691 ± 0.01	0.0813 ± 0.01*	0.0559 ± 0.01*
Femur-Proximal	0.183 ± 0.02	0.179 ± 0.02	0.117 ± 0.01*	0.105 ± 0.01*
Femur-Shaft	0.108 ± 0.02	0.112 ± 0.02	0.044 ± 0.01*	0.0561 ± 0.01*
Femur-Distal	0.214 ± 0.02	0.182 ± 0.03	0.119 ± 0.02*	0.0989 ± 0.02*
Femur-Marrow	0.282 ± 0.05	0.158 ± 0.05†	0.131 ± 0.03*	0.0645 ± 0.02†*
Tibia-Proximal	0.207 ± 0.02	0.163 ± 0.01	0.102 ± 0.02*	0.108 ± 0.01*
Tibia-Shaft	0.0673 ± 0.01	0.0732 ± 0.01	0.0126 ± 0.004*	0.0194 ± 0.01*
Tibia-Distal	0.0419 ± 0.01	0.0249 ± 0.01	0.0178 ± 0.01*	0 ± 0*
Fibula	0.0179 ± 0.01	0.0385 ± 0.01	0 ± 0*	0 ± 0*

Values are means ± SE in ml • min^{-1} • 100 g^{-1} • $mmHg^{-1}$. † Aged mean is different from corresponding young mean, *P<0.05*. * 10 minute head-up tilt mean is different from corresponding baseline mean, *P<0.05*.

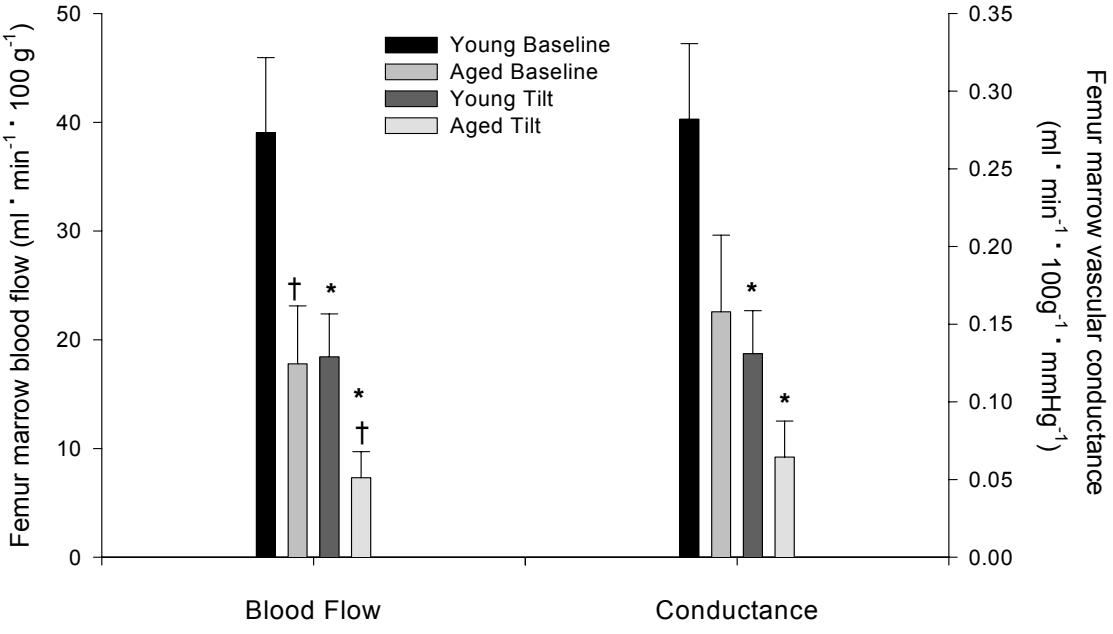

Fig. 3. Blood flow and vascular conductance to the femur marrow of young (4 mos.) and aged (24 mos.) rats during baseline quiet standing (0° tilt) and after 10 minutes of head-up tilt (70° tilt). Values are means ± SE. † Aged mean is different from corresponding young mean, *P<0.05*. * 10 minute head-up tilt mean is different from corresponding baseline mean, *P<0.05*.

DISCUSSION

The purpose of this study was to test the hypothesis that there is a diminished ability to maintain MAP during orthostasis in aged Fischer-344 rats, and to identify whether tissue perfusion and the inability to lower vascular conductance could contribute to the lowering of arterial pressure. Our data support this hypothesis in that the aged rats failed to maintain MAP during the 10 minute tilt (Fig. 2) and vascular conductance was higher during tilt in the old animals in the adipose tissue (Table 2), testes (Table 4), adrenal medulla (Table 4) and white gastrocnemius and adductor muscles (Table 6). However, contrary to our hypothesis, the vast majority of regional blood flows and vascular conductances decreased to the periphery (hind-limb) similarly in both the young and aged animals. The most notable finding of this study was the decrease in blood flow and vascular conductance to both the bones and bone marrow of the hind limb in the aged animals during baseline measurements.

Because the hypothesized increase in blood flow and vascular conductance during tilt was not evident in most tissues with aging, a reduced cardiac output must be the underlying cause of the attenuation in MAP during head-up tilt in the aged animals. Based on Ohm's law applied to the cardiovascular system, cardiac output can be expressed as:

Cardiac Output = Arterial Pressure X Total Vascular Conductance

If arterial pressure is decreased and vascular conductance is not changed, then cardiac output must be reduced. There is evidence of a reduction in cardiac output with advancing age in rats (1, 78, 97, 98, 110) and humans (29, 53, 56, 61, 102), as well as in

advancing age with tilt (56), which would explain the reduction in MAP in the aged animals.

Past research has shown that the elderly maintain stroke volume with advancing age (53, 67, 102). This is due to slower calcium cycling which causes a prolonged contraction. There is also an augmented late filling of the ventricle which is due to a stronger atrial contraction. In the younger heart the atria act as conduits and do not play a major role in ventricular filling, but because of the increased myocardial stiffness, the atrial contraction plays a larger role in ventricular filling in the aged heart. Based on the equation:

Cardiac Output = Heart Rate X Stroke Volume

if stroke volume is maintained and heart rate is decreased this would further support the notion that cardiac output was reduced with age and could possibly be the primary underlying factor in the drop in MAP in the aged rats.

The attenuated heart rate response during tilt in the old animals is consistent with a reduction in cardiac output (Fig. 1). This attenuation in the heart rate response or cardioacceleration with advancing age during tilt is evident in previous studies (42, 67, 90, 94, 102) and could also help to explain the inability of the aged animals to maintain MAP (Fig. 2). This decreased ability of cardioacceleration to buffer against a reduction in MAP is most likely due to reduced β adrenergic responsiveness and/or a attenuation in the cardiovagal baroreflex sensitivity. With age there is a decline in response to ß-adrenergic stimulation with respect to HR, vascular smooth muscle cells, and myocardial contractile response (89). Increased plasma norepinephrine levels lead to a diminished

ß-adrenergic affinity of the receptor to the agonist and the coupling of the receptor to the catalytic subunits decreases (G-protein and adenylate cyclase) (53).

It is has been suggested that with aging, cardiovagal sensitivity is diminished (44, 71). This could be due to a reduction in carotid arterial compliance with age, which would limit arterial deformation in response to changes in MAP (71). Numerous vascular beds in both the human and animal models have been shown to increase stiffness with advancing age (8, 29, 36, 68, 69). While Fischer 344 rats are free from atherosclerosis, their vessels have been shown to become less elastic with age (unpublished observation) due possibly to a shift in collagen isoforms, loss of elastin, and/or collagen cross-linking (29). For example, Zhang et al. (113) showed a decreased distensibility of mesenteric arteries from aged female Sprague-Dawley rats in response to stepwise increases in pressure.

Blood flow and vascular conductance were largely maintained with aging. This was contrary to the idea that a reduction in MAP would be associated with an inability to maintain or reduce blood flow and vascular conductance due to a diminished myogenic response, which has been shown skeletal muscle resistance vasculature of aged Fischer 344 rats (73). There are many compensatory hemodynamic mechanisms that could compensate for an attenuated myogenic response during head-up tilt. These include, 1) increased sympathetic outflow, 2) a greater responsiveness to vasoconstrictor stimuli, and 3) increased vessel stiffness. Any of these alone or in combination could account for the similar hemodynamic results between the young and aged rats.

Evidence for increased sympathetic outflow with aging is evident in both human and animals (29, 53). According to Folkow and Svanborg (29) plasma norepinephrine concentrations increase 10-15% per decade in the mature adult. Furthermore, Kohrt et al. (52) found that plasma norepinephrine levels increased in response to 10 min of quiet standing in 106 healthy aged men and women when compared to their younger counterparts, whereas Taylor et al. (102) saw no increase in plasma norepinephrine levels during both quiet standing and lower body suction in their aged subjects compared to their young subjects. Meanwhile, work by Dinenno (23, 24, 25) and colleagues has shown that there is a significant increase in efferent sympathetic nerve activity in the leg with increasing age in healthy adult humans. While evidence for an increase in plasma norepinephrine concentrations during standing is somewhat mixed, the preponderance of research shows plasma catecholamine concentrations are elevated with aging (88, 89).

Another compensatory hemodynamic mechanism would be a greater responsiveness to sympathetic stimulation in the aged. A greater reactivity to norepinephrine would help to compensate for a diminished myogenic response. While there is evidence for increased basal levels of sympathetic vasoconstrictor nerve activity (23, 24, 25), the evidence for greater sensitivity to sympathetic stimulation is lacking. Muller-Delp et al. (73), using an isolated vessel model, showed no significant difference to increasing doses norepinephrine in either the gastrocnemius or soleus muscle arterioles. Furthermore, Wilson and colleagues (112) found cutaneous vasoconstrictor responsiveness to infused norepinephrine to be diminished in elderly subjects when

endogenous release of norepinephrine was blocked. While this might be a factor in overcoming a diminished myogenic response, the research is inconclusive.

Arguably two of the most important regions in the body regulating total vascular conductance in the maintenance of mean arterial pressure during an orthostatic event are the hind-limbs (periphery) and the splanchnic region (86). In the present study the aged rats demonstrated a diminished splanchnic blood flow during the level baseline condition and, unlike the young rats, failed to reduce blood flow at 10 min of head-up tilt (Figure 4). These results are counter to previous data in which elderly human subjects who were exposed to head-up tilt increased splanchnic vascular resistance significantly higher and had a greater reduction in splanchnic blood flow than their younger cohorts (67). There is evidence of increased stiffness in mesenteric arteries in rats (113). A reduction in the elasticity of mesenteric arteries could explain the lower blood flows and vascular conductance across both conditions in this study. Mesenteric resistance arteries in rats also have been shown to increase wall thickness and medial cross sectional area with age, which was positively related to increases in pulse pressure (72). Although room conditions were kept consistent in regards to temperature there is the possibility that the combination of extended time (~20 min.) in the tilting apparatus, in which the aged rats had less “free space” in combination with an increased adipose tissue in the aged in the Fischer 344 rat (19), caused an increase in temperature in the aged rats above that of the young rats. There is evidence of greater splanchnic vascular conductance in the elderly in response to heat in humans and animals. In rats, for example, Kenney et al. (47) found that sympathetic nerve discharge to renal and splanchnic tissues increased in

young and mature Fischer 344 rats, but not in senescent rats, in response to increases in body temperature from 38° to 41°. In another study, Kenney and Musch (48) found that blood flow responses to increased temperatures were reduced to the splanchnic and renal tissues in young and mature rats but did not change in the senescent animals. They also found no change in blood flow to the muscles of the hind-limb across all three age groups. In humans it has been shown that the decrease in splanchnic blood flow exhibited in young men during exercise in a warm environment was attenuated in aged men (49). Subsequent studies by Minson and colleagues (66, 67) support the idea that there is a reduced splanchnic response in regards to both increasing vascular resistance and decreasing blood flow in response to heat. This would offer the possibility of a head-up tilt-related decrease in splanchnic vascular conductance being off-set by a heat related rise in splanchnic vascular conductance in the aged animals.

Several splanchnic tissues showed attenuated perfusion with age. Blood flow and vascular conductance to the spleen were significantly lower in the aged animals and

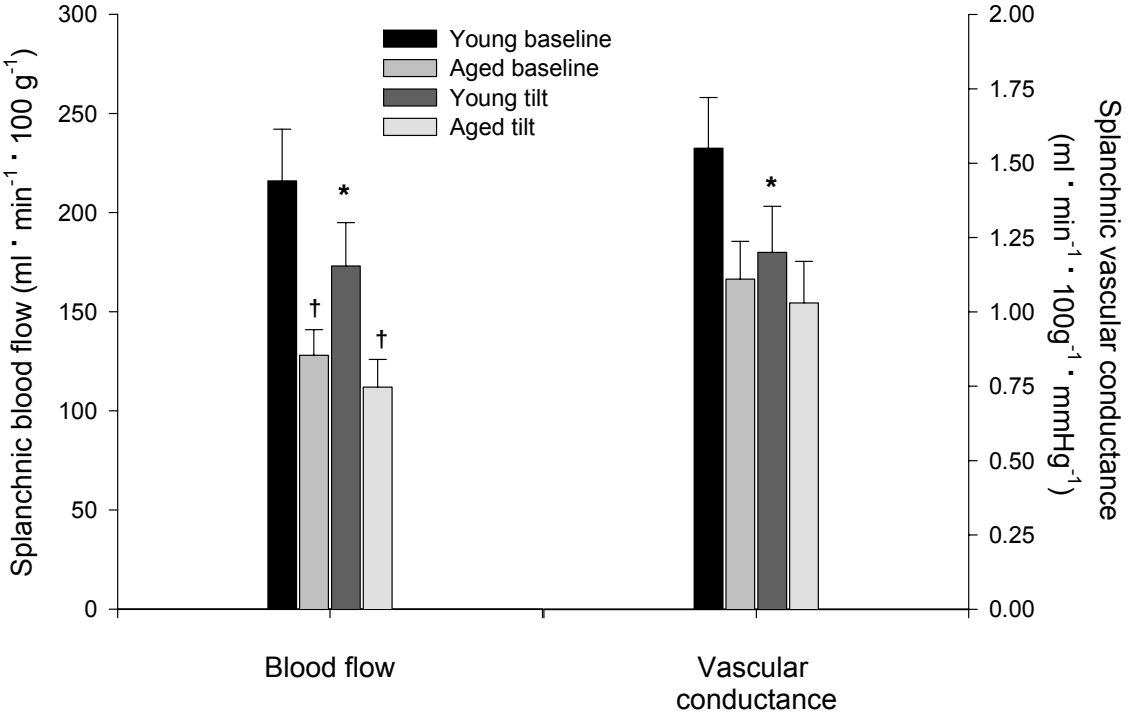

Fig. 4. Blood flow to and vascular conductance in splanchnic tissues of young (4 mos.) and aged (24 mos.) rats during baseline quiet standing (0° tilt) and after 10 minutes of head-up tilt (70° tilt). Values are means ± SE. † aged mean is different from corresponding young mean, *P<0.05*. * 10 minute head-up tilt mean is different from corresponding baseline mean, *P<0.05*.

were reduced with tilt only in the young animals. Both Delp et al. (19) and Musch et al. (75) have reported spleen blood flows to be reduced with age. The lack of significant reduction in blood flow during tilt in the aged most likely is a reflection of diminished reserve blood flow capacity which is normally seen in splanchnic organs (86). Blood flow to the mesentery, large intestine and rectum were diminished with age, and there was a significant decrease in blood flow to the mesentery with tilt. While the previous two studies of Delp et al. (19) and Musch et al. (75) did not report significant differences

in flow in these tissues, both showed a trend for reduced perfusion. Both also reported reductions in flow to the large intestines, and while Musch et al. (75) did not report data on the mesentery, Delp et al. (19) did find similar reductions with age to that tissue as well.

In the present study both blood flow and vascular conductance to the combined hind-limb tissues were significantly reduced during the tilt condition across the two age groups (Fig. 5). This goes against evidence from the current literature, as well as our hypothesis, that an increased hind-limb blood flow and vascular conductance would help account for a decrease in mean arterial pressure. In aged humans during head-up tilt, Smith et al. (94) showed increases in leg volume, while attenuated forearm vascular resistance (67) has also been demonstrated. However, in human studies involving head-up tilt, the subject is positioned on his/her back and leg muscle activation in minimized. In the present study the rats are supported by their limbs during both conditions so that there is a possibility of static muscle contraction during head-up tilt that might cause a decrease in total hind-limb blood flow and serve to keep blood from pooling in the venous circulation of the hind-limb. Recent studies also indicate that the increase or decrease in responsiveness to sympathetic stimulation depends on the area of the body

studied. For example, Minson et al. (67) showed that while there was an increased vasoconstrictor response in the splanchnic region, there was also a diminished response in the forearm of elderly subjects compared to their younger counterparts during both tilt and heat stress. Proctor et al. (81) also showed regional specificity to reductions in vascular conductance in elderly subjects. Vascular conductance was measured after 10 minutes of arterial occlusion in the forearm and calf of men 20 – 79 yr. of age. Peak limb vascular conductance responses in the calf of the aged subjects closely matched the responses of the young, while the responses in the forearm were significantly different between young and aged subjects. Furthermore, Muller-Delp et al. (73) demonstrated that there were no significant differences in the responses to norepinephrine in the hind-limb vasculature between young and aged Fischer 344 rats. So while certain areas of the body have demonstrated an increase in vascular conductance with age, other areas have shown either no change or a concomitant decrease in vascular conductance.

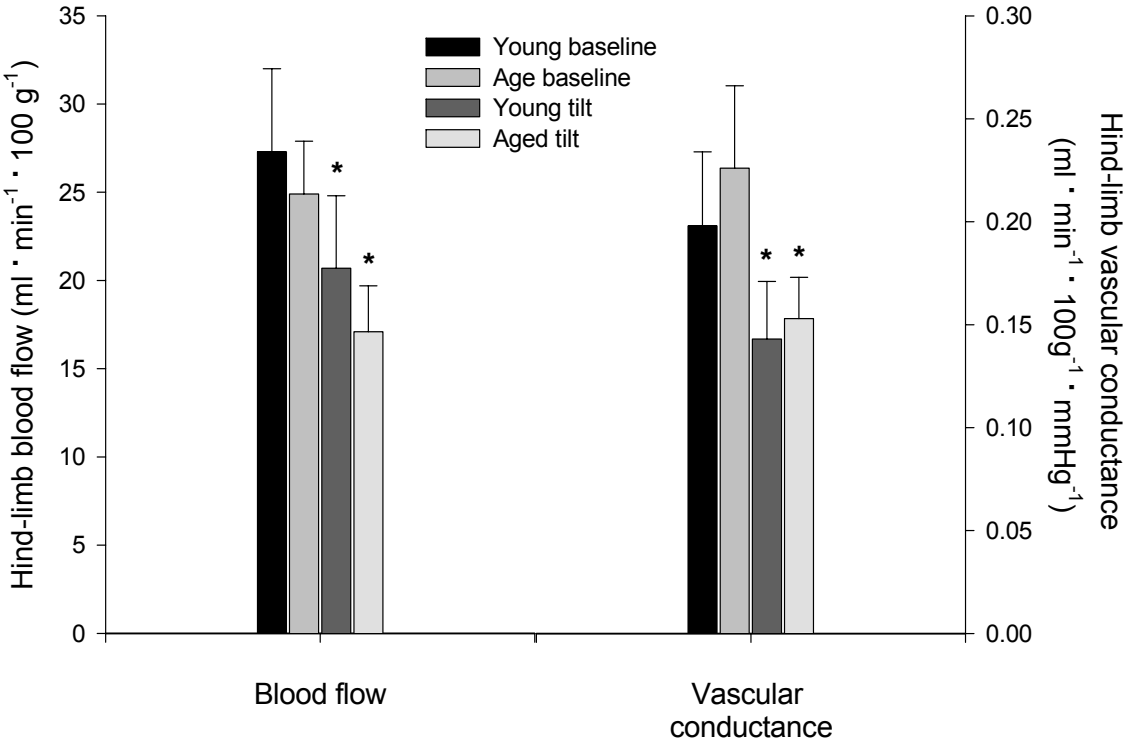

Fig. 5. Blood flow to and vascular conductance in hind-limb tissues (bones, muscles, skin, and subcutaneous fat) of young (4 mos.) and aged (24 mos.) rats during baseline quiet standing (0° tilt) and after 10 minutes of head-up tilt (70° tilt). Values are means ± SE. * 10 minute head-up tilt mean is different from corresponding baseline mean, $P<0.05$.

While total hind-limb blood flow and vascular conductance were similar between young and aged rats, individual tissues of the hind-limb did show some age-related differences. Hind-limb skin and subcutaneous fat blood flow and vascular conductance in the young were decreased with tilt while there were no differences in the aged animals. The baseline values of blood flow and vascular conductance to these tissues were significantly different between the age groups as well. The inability of the aged to decrease peripheral vascular conductance is well established. Specifically, Minson et al. (67) showed an attenuated ability in the elderly to decrease forearm vascular

conductance in response to head-up tilt and head-up tilt with heat. Furthermore, Wilson et al. (112) showed a decreased responsiveness to norepinephrine in the cutaneous circulation of aged subjects.

Results from the muscles of the hind-limb yielded very few significant differences with age or tilt. The soleus was the only hind-limb muscle to reduce blood flow with tilt. This was to be expected as the rat soleus muscle is comprised mainly of type I (slow-oxidative) muscle fibers (18, 99) and it is active during normal standing as a postural muscle. In the tilt position the hind-limb muscles could potentially be unloaded, which would explain the differences found between the two conditions in the soleus mucles. The white portion of the gastrocnemius muscle showed an increase in vascular conductance during with age across both conditions. The resistance arterioles responsible for perfusion of this portion of the gastrocnemius muscle have a diminished myogenic response in aged Fischer 344 rats (73). In the arterioles from the white portion of the gastrocnemius, spontaneous tone was attenuated compared to soleus muscle arterioles. Additionally, in a study by Muller-Delp et al. (74) that reported endothelium-dependent vasodilation to be diminished in isolated arterioles from soleus muscle of aged rats, there was no attenuation in endothelium-dependent vasodilation in the white portion of the gastrocnemius muscle in aged rats. Vascular conductance to the adductor muscle group (adductors longus, magnus, brevis, and gracillis) was also elevated in the aged rats. These muscles, like the white portion of the gastrocnemius, are comprised primarily (~85%) of type II muscle fibers (18), which could explain the similar results between the muscles.

The present study demonstrates that there is a dramatic decrease in hind-limb bone and bone marrow perfusion with old age (Table 7). Previous studies with hind-limb unloaded rats have linked decreases in bone blood flow with loss of bone mineral density (7, 14). Therefore, the decrease in bone and marrow perfusion reported in the present study could likewise be associated with the osteoporosis often occurring with old age. For example, reductions in bone material properties with aging have been reported in male rats of the same ages as the rats of the present study (6 and 24 months) (50). Likewise, studies of bone from human cadavers have yielded similar changes in bone material properties with aging as those reported in the rat (10, 28). Therefore, these studies indicate that osteoporosis and the decline in rat bone material properties appears to be similar to that which occurs in human bone with aging.

Several mechanisms could serve to link changes in bone perfusion with the skeletal decline associated with aging. The first is the effect that alterations in blood flow may have on interstitial fluid flow. It has been proposed that alterations in bone interstitial fluid flow may influence bone remodeling (7). Interstitial fluid flows radially through cortical bone, driven by a transmural pressure gradient between the vasculature of the endosteal surface and the periosteal lymphatics (84). Mechanical loading of bone causes fluid flow through cancellous bone and the lacunacanalicular network of cortical bone, exacerbating flow-induced shear stress imposed on surface bone cells (93). The shear stresses generated by bone interstitial fluid flow appear to be of similar magnitude to those occurring at the blood-vascular endothelium interface (85). Bone cells respond to fluid shear forces in a manner similar to vascular endothelial cells, by generating

autocrine or paracrine signals that modulate remodeling activity (43, 84). Results from the present study of a diminished bone perfusion with aging may have direct effects on interstitial fluid flow, and consequently, bone formation by reducing capillary and sinusoid fluid filtration.

A second mechanism through which altered bone perfusion may be linked to skeletal remodeling with old age is via a vascular mechanism (79). Based upon the juxtaposition of blood vessels and bone cells, it was first proposed in 1930 that the bone's vascular network might be an active mediator of skeletal remodeling (41). More recently, it has been demonstrated that blood vessels are located in the basic multicellular units containing osteoclasts and osteoblasts that carry out bone remodeling (79). In addition, these vessels are located no more than 100 μm from the site of each unit's remodeling activity (79). Vascular endothelial cells release substances in response to changes in fluid flow and shear stress that may act directly on bone cell populations or serve as paracrine modulators of bone cell activity within the basic multicellular unit (29, 46, 60, 79). Specifically, NO and prostacyclin (PGI_2) are potent vasodilators that are, in many tissues, released by the vascular endothelium in response to blood flow, and correspondingly, intravascular shear stress (77). The effects of NO to increase bone formation and decrease bone resorption are well established (58, 60, 85, 107), and PGI_2 has been shown to exert a direct inhibitory effect on osteoclasts (13, 83). Therefore, this raises the possibility that old age-related decreases in blood flow and shear stress alter vascular endothelial cell release of these agents, which could subsequently modify the focal balance between osteoblast and osteoclast activity.

All skeletal samples tested for blood flow had marrow intact except for the femoral shaft, where perfusion of the marrow was measured as a separate compartment. Therefore, some of the age-related changes in marrow blood flow and composition may be a strong determinant of changes in bone tissue blood flow. In humans, hematopoietic marrow progressively shrinks in size during maturation, with much of it replaced by fatty marrow, particularly in the long bone shafts (106). Blood flow to fatty marrow is approximately one-third of that to hematopoietic marrow (33); therefore, any skeletal site containing a greater proportion of fatty marrow will have lower blood flow. Whether other alterations, such as changes in bone resistance artery vasodilator or vasoconstrictor properties, also occur with old age and contribute to the decrease in bone blood flow remain to be determined.

While measured changes to overall tissue blood flow and vascular conductance could not explain the difference in MAP between the young and old animals, there was a reduction in blood flow and vascular conductance that was seen in the left and right kidneys of young but not in the aged animals. Blood flows and vascular conductance to both the left and right kidneys were similar to spleen flows in that they were both lower in the aged animals at baseline and there was little to no change during head-up tilt while the young rats significantly lowered both blood flow and vascular conductance during head-up tilt. These results are in agreement with Minson and colleague's study (67) and consistent with other studies that show an age related decline in renal blood flow in both humans (49) and rats (75).

There have been relatively few studies investigating the effect of age on regional blood flow distribution in animals (34, 35, 65, 105) and even fewer that included large tissue samples in conscious rats (19, 75). In the latter two studies there were some slight variations in the results when compared to the present study. For example, Musch et al. (75) showed differences in blood flow per unit mass between 6 to 8 month and 27 to 29 month old Fischer-Brown Norway rats in the spleen and kidneys. This study did not report results for fat, reproductive, or bone tissues. Meanwhile, the study by Delp et al. (19) found age-related reductions in tissue perfusion between 6 month and 24 month old Fischer 344 rats only in the spleen, prostate gland, and thyroid and parathyroid glands. The current results show age-related reductions in blood flow per unit mass to subcutaneous fat, spleen, mesentery, large intestine and rectum, prostate, adrenal cortex, kidneys, hind foot, distal femur, femur marrow, proximal tibia, distal tibia, and urinary bladder. The differences between the current study and that of Musch et al. (75) could be a result of the conditions under which the measurements were taken. Blood flow measurements were taken on a treadmill prior to an exercise bout and after the rats had followed a 2 to 3 week acclimatization period of treadmill running. The primary difference is that because the animals were not restrained as in the current study and had room to move during the resting measurements, the resting flows could have involved some movements which may have altered blood flow measurements. This could also help explain the differences with the study by Delp et al. (19) in which resting measurements were take in a cage that did not confine the movements of the rats. Another factor that could have affected the resting results from the study by Musch et al.

(75) is that there is evidence of both anticipatory heart rate (64) and blood flow (2, 3, 4, 54, 55) responses to exercise in humans and rats which could have altered tissue flows.

SUMMARY AND CONCLUSIONS

The purpose of this study was to determine the effects of old age on mean arterial pressure, heart rate, and regional cardiac output distribution during head-up tilt in conscious rats. Both an increase in the population over the age of 65 and the increased incidence of orthostatic hypotension with advancing age warrants further research in the area of alterations in cardiovascular function in the elderly. The present findings support the concept that orthostatic hypotension occurs with advancing age, and is the primary result of an attenuated heart rate response and a putative reduction in cardiac output, but not an inability to decrease total vascular conductance. Not only were the aged rats unable to increase heart rate upon acute tilt, there was still a lower heart rate after 10 min of head-up tilt. Furthermore, the aged rats were able to decrease both blood flow and vascular conductance to the hind-limb in response to head-up tilt similar to the young. These results provide evidence that the initial maintenance of MAP in aged rats during head-up tilt occurs through decreased regional blood flow and vascular conductance, and that the fall in pressure is attributable to diminished cardiac performance. In addition this is the first study to demonstrate a decreased bone vascular conductance in both young and old rats during head-up tilt. The decrease in bone blood flow to the hind limb could have profound implications related to the reduction in bone mineral density with aging.

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VITA

Name: Michael Wiechmann Ramsey

Address: P.E.X.S.
East Tennessee State University
P.O. Box 70654
Johnson City, TN 37614

E-mail: ramseym@etsu.edu

Education: B.S. Kinesiology, Sam Houston State University, 1993
M.A. Education, Sam Houston State University, 1995
Ph.D. Kinesiology, Texas A&M University, 2005